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Implementation of a capillary array electrophoresis instrument

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Abstract

A capillary array electrophoresis (CAE) apparatus capable of running and analyzing DNA samples in 48 capillaries simultaneously has been constructed. The capillaries are individually replaceable, and sieving buffer can be easily pumped in and out of the capillary array as necessary. Samples are injected electrokinetically from polymerase chain reaction (PCR, Hoffmann-LaRoche, Nutley, NJ, U.S.A.) tubes arranged in a 6 × 8 format and are detected by laser-induced fluorescence. Data analysis software has been developed for semiautomatic analysis, including peak finding and DNA fragment sizing. The system represents a robust apparatus for the rapid and convenient analysis of DNA fragments in a high-throughput environment.

Recent work has shown that CE is a potentially powerful tool for mapping and sequencing large segments of DNA.[1-24] The small cross-section and high surface area of the capillary result in excellent resolution and rapid separations compared to slab gel formats.[22-24] Single base-pair resolution for DNA sequencing is possible up to at least 500 base pairs and can be achieved in <2 hr.[1-9,13] In addition, the separation performance of the capillary can be manipulated to resolve larger fragments of DNA for mapping or genetic typing applications.[14-18] Furthermore, the capillary format lends itself well to process automation.

Traditional CE has been hampered by the low throughput of instruments accommodating only a single capillary. Recently, this limitation has been overcome with the advent of capillary array electrophoresis (CAE), in which many capillaries are run and analyzed simultaneously.[1-11] Reported here is the development of a 48-CE apparatus that combines convenient hardware interfaces for sample and sieving buffer injections with sensitive fluorescence detection and sophisticated image analysis software. Using hydroxyethylcellulose (HEC) as a sieving buffer, the 48 capillaries can be filled and equilibrated under run conditions and the DNA samples injected and detected in <60 min. The system employs laser-induced fluorescence (LIF) detection through a confocal optical design.[1-3] Thiazole orange (TO) can be included in the sieving buffer to detect ds-DNA or, alternatively, sample unknowns and internal DNA size standards can be end-labeled with different fluorophores. The instrument design also lends itself to further enhancements, including higher levels of sample throughput and increased automation.

Results and discussion

The growth of DNA analysis for forensic, therapeutic, and genome project applications has driven the need for improved experimental platforms. Improvements over current technology are required to provide shorter analysis times, higher sample throughput, increased automation, and improved analysis software. One of the emerging fields in DNA-separation instrumentation is CAE.[1-15] This approach can provide the fast, high-resolution separations possible with traditional CE coupled with the sample multiplicity of slab gel formats. However, the evolution of CAE from laboratory demonstrations to fully integrated, cost-effective, robust, and convenient instrumentation has been slow. For example, it is not practical for both cost and

Indexing terms

Capillary array electrophoresis, DNA fragments, semiautomatic analysis

Abbreviations

CAE, capillary array electrophoresis; HEC, hydroxyethylcellulose; LIF, laser-induced fluorescence; TO, thiazole orange; PCB, printed circuit board; PMT, photomultiplier tube; RQE, relative quantum efficiency; Tris-EDTA, Tris-ethylenediaminetetraacetic acid; TBE, Tris-borate-EDTA; TEMED, *N,N,N'N'*-tetramethylethylenediamine; APS, ammonium persulfate

convenience reasons to use prefilled capillaries that must be replaced after a few runs (as is the case with cross-linked polyacrylamide-filled capillaries). Although a number of groups have developed replaceable sieving buffers for CE,[18–20,27–40] no group has yet described a useful interface and process for pumping these viscous solutions into and out of a large capillary array. The convenient and reliable injection of large numbers of samples has also not been addressed. We have developed a more complete CAE system to address these and other performance issues.

Electrophoresis subsystem

An overview of the electrophoresis system is shown in *Figure 1*. On the cathode side, the capillaries are arranged in a 6 × 8 array (half multiple-well plate). The cathode manifold (*Figure 2*) consists of a printed circuit board (PCB) on which 48 platinum electrodes have been traced and bent down perpendicular to the PCB to create the 6 × 8 array. Capillaries are held in place by passing the glass through short sections of small-diameter Tygon™ (McMaster-Carr, Los Angeles, CA, U.S.A.) tubing mounted in holes drilled through the PCB adjacent to each electrode. The capillary tips are situated in close proximity to the electrode ends. Sample and buffer tubes are held in a plastic rack and placed on a stage platform. The stage raises the sample or buffer tubes to immerse the ends of the electrodes and capillaries. All samples are electrokinetically injected simultaneously in 5–15 sec. Sample and buffer tubes can be exchanged by replacing the rack, a step that can be accomplished in <5 sec. Information about the electrical current in each capillary is collected and stored during the electrophoresis run. This is accomplished by placing a 100-kΩ resistor in series with each electrode on the cathode side. Voltages across these resistors are measured and passed to a 12-bit 48-input data acquisition card.

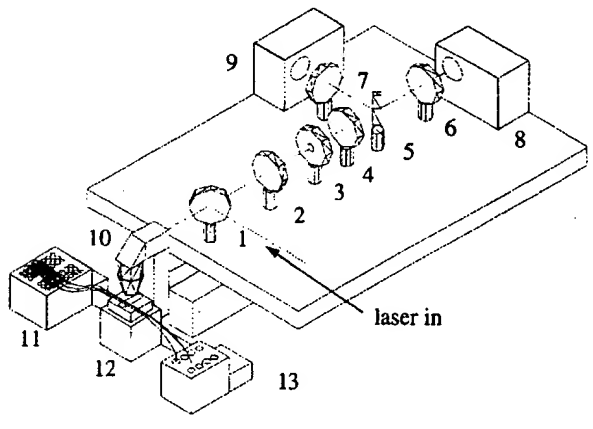

FIGURE 1 *Schematic of optical and electrophoresis subsystems. Components: 1) primary beamsplitter, 505DRLP; 2) laser filter, 510EFLP; 3) achromat lens; 4) confocal spatial filter; 5) secondary beamsplitter; 6) bandpass or longpass filter; 7) bandpass or longpass filter; 8) PMT 1; 9) PMT 2; 10) objective mounted to translation stage; 11) cathode manifold; 12) capillary detection window mount; 13) anode pressure manifold.*

In the center of the unit, the capillaries are threaded through a detection area (*Figure 3*), where the windows of the capillaries are held in a plane perpendicular to the exciting laser beam. The window holder is a ceramic piece with v-grooves machined along it. Two top pieces of ceramic act as clamps to hold the capillaries in position. Capillaries slide easily in and out of the grooves, permitting quick replacement of individual capillaries with no optical alignment required. The window holder is mounted to a vertical stage, allowing the capillary array to be moved into the proper focal plane for the microscope objective. Once the window holder has been positioned vertically, no further adjustments are required, even if capillaries are replaced in the system. It takes approximately 30 min to completely install a full array of 48 capillaries.

At the anode chamber, the capillaries are separated into eight groups of six (groups A–H, capillaries 1–6 in each group, *Figure 4*). Each group has its own platinum electrode. The capillaries are inserted into the anode chamber through pieces of Tygon tubing. For each group, there is a clamp device that serves to create a pressure seal around the capillary-tubing interfaces (not shown in photograph for clarity). This permits pressure up to 100 psi to be applied to the anode chamber to pump solutions or sieving buffer through the capillaries. The 100-psi limit of the design restricts this manifold to sieving buffers suitable for dsDNA separations. Separation of the capillaries into the eight groups also makes it possible to run eight different sieving buffers simultaneously. One can therefore multiplex eight different electrophoresis conditions with six capillaries for each condition. This flexibility is extremely powerful for method development and experimental optimization. Solutions are held in standard microcentrifuge tubes in a plastic rack placed on a stage platform similar to that on the cathode side. The stage raises the tubes to immerse the ends of the capillaries and electrodes and locks in place to create a pressure seal. A switch is mounted in the unit to control the application of pressure, supplied by an N_2 cylinder.

The entire assembly is housed in a light-tight, thermally insulated chamber equipped with safety interlocks for the high voltage supply, the photomultiplier tube (PMT) power supplies, and the laser shutter. Three internal fans provide ample convection over the capillary array to ensure efficient heat dissipation and thermal equilibration during a run. Thermal stability during a run and uniformity around the capillary bed are maintained to better than ±0.5 °C.

Optical subsystem

The instrument optical design (Figure 1) is based on a system described by Mathies et al.[1–3] Light from a

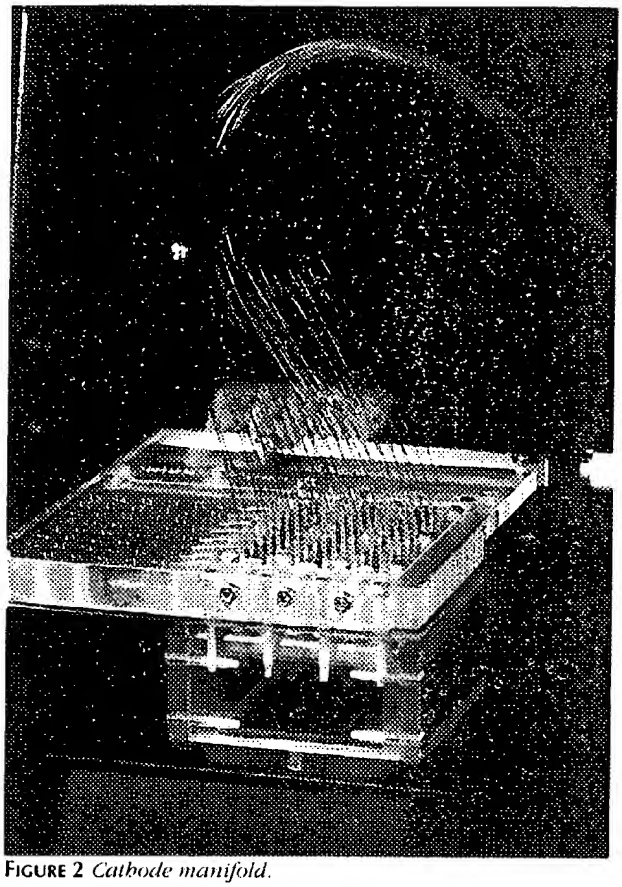

FIGURE 2 *Cathode manifold.*

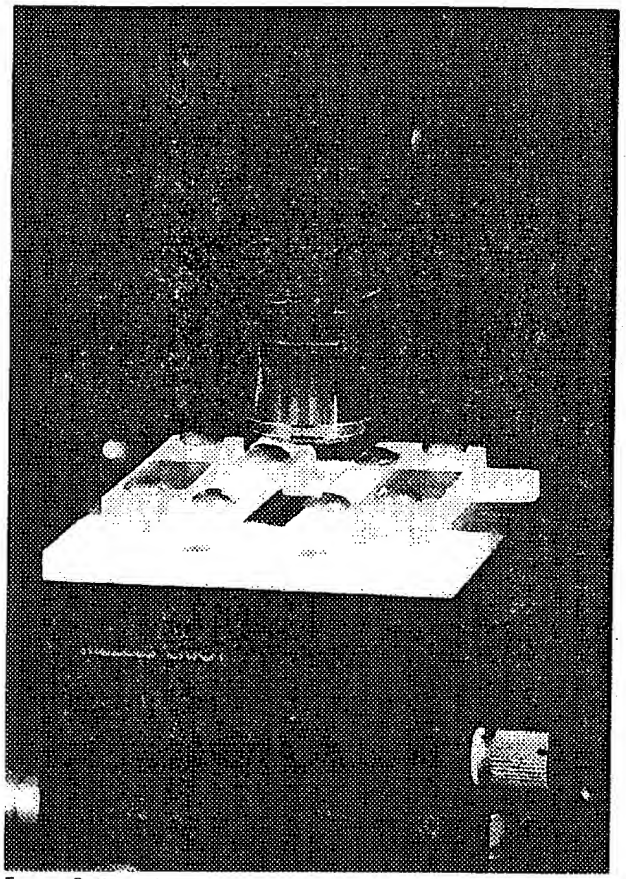

FIGURE 3 *Detection area.*

20-mW Ar^+ laser is passed through a beam expander and reflected off the primary dichroic beamsplitter (505 DRLP, dichroic reflective long-pass). The light then passes through a microscope objective that focuses the excitation light onto the plane of the capillary array. The objective is mounted to a translation stage and driven over the capillary bed, sequentially illuminating each capillary. The scan rate is 1 Hz. In contrast to previous designs,[1-5] the capillaries remain fixed during the scan while the objective moves. The fluorescence emission is collected by the same objective lens and travels back through the primary beamsplitter. An achromatic lens focuses the fluorescent signal onto a spatial filter, and the light is then split into two optical detection channels by a secondary beamsplitter. Additional long-pass or band-pass filters can be inserted after the secondary beamsplitter to enhance spectral separation between the two channels. Detection is accomplished by two PMTs. The secondary beamsplitter and final optical filters are easily accessible from the top of the unit and are user changeable to allow the use of any pair of 488-nm excitable fluorophors. Regardless of the filter sets used, there is always some degree of spectral cross-talk between channels. ArrayQuant™ (Molecular Dynamics, Sunnyvale, CA, U.S.A.) software includes a feature that

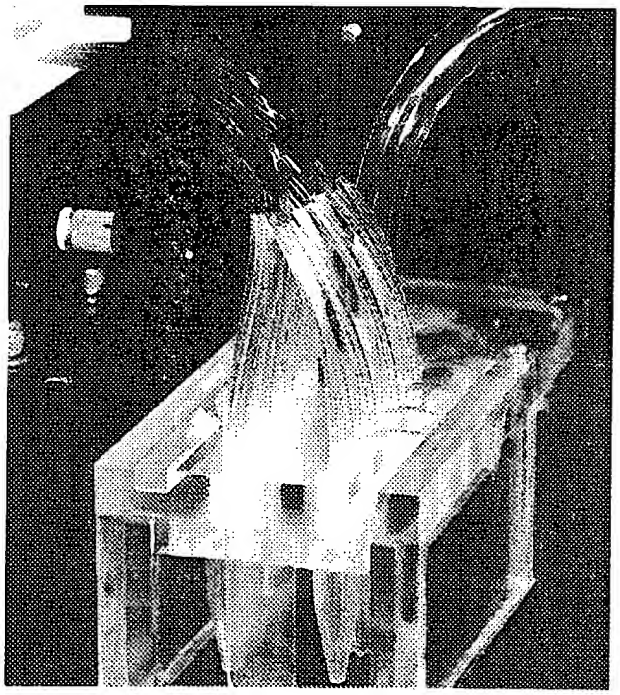

FIGURE 4 *Anode chamber.*

allows the user to subtract out the remaining cross-talk from each channel.

The capillary signals are sampled unidirectionally at a 38-kHz sampling rate for each channel. The PMT signals are passed through a preamp, filtered, and digitized by a 12-bit, 16-input data acquisition card. The image spatial resolution is 37.5 μm. The temporal resolution is 1 Hz, corresponding to the scan rate of the translation stage. Signal response as a function of fluor concentration (*Figure 5*) was measured with BODIPY505/515™ (Molecular Probes, Eugene, OR, U.S.A.) and found to be linear over 3.5 orders of magnitude (R^2 = 1.000). Signal-to-noise (S/N) response as a function of signal level is shown in *Figure 6* for three different PMT voltage settings. To determine the optical detection limit of the system, capillaries were scanned while flowing methanolic solutions of the BODIPY dye

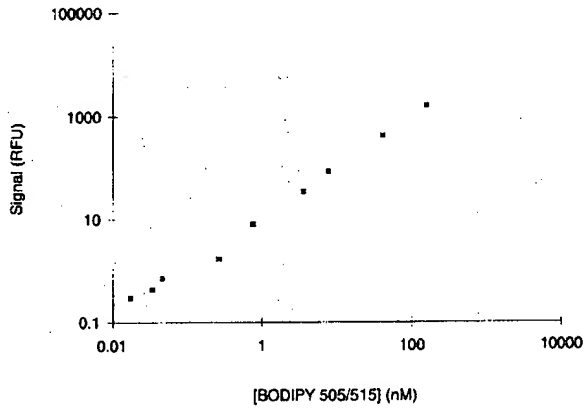

FIGURE 5 *Plot of signal (in relative fluorescence units, RFU) versus fluor concentration. Capillaries filled with flowing BODIPY510/515™/methanol solutions. PMT voltage = 400 V; optical filter 520DF30; laser power 5.7 mW at the sample. Linear fit to the data shows R^2 = 1.000.*

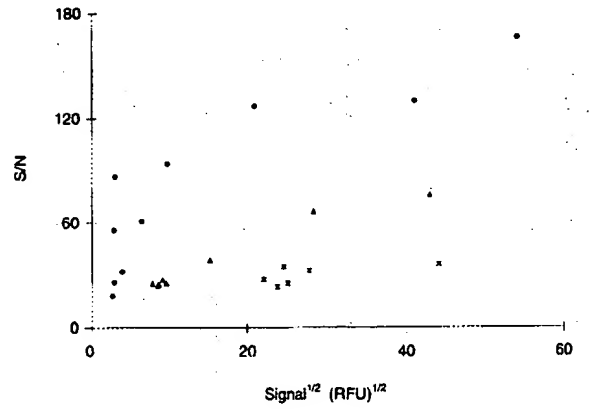

FIGURE 6 *Plot of signal/noise versus (signal)$^{1/2}$ at various PMT voltages. Capillaries filled with flowing 2 pM-5 mM BODIPY505/515/methanol solutions; laser power 5.7 mW at the sample; optical filter 520DF30. PMT voltages: circles, 400 V; triangles, 600 V; stars, 800 V.*

at concentrations down to 10^{-12} *M*. The relative fluorescence values at the center of the capillaries were plotted as a function of BODIPY505/515 concentration. Under these conditions, the lower detection limit, defined at S/N = 1, is approximately 9×10^{-21} moles of BODIPY505/515 per pixel. The BODIPY family of dyes is known to be resistant to photobleaching and triplet saturation.[25] Evidence of fluor saturation was not seen at laser powers up to 23 mW. It was, however, quite easy to saturate fluorescein under the same conditions (data not shown).

Relative quantum efficiency

The relative quantum efficiency (RQE) of the instrument is the ratio of the fluorescent photons emitted from the sample to the photons detected. The following is a calculation of RQE using BODIPY505/515 at the limit of detection (LOD = 30 p*M* BODIPY505/515).

Excited fluors

The number of fluorescent photons emitted from the capillary during 1 pixel can be estimated by calculating the power of the fluorescent emissions, which can be estimated by:

$$W_{out} = A^{*}P^{*}QE \qquad (1)$$

where A, the absorption of the excitation light, is given by εbc (ε = dye extinction coefficient at 488 nm [98,000], b = path length [100 μm] and c = dye concentration at the detection limit [30 p*M*]); P = laser power at the sample (5.7 mW); and QE = dye quantum efficiency (0.94). Multiplying W_{out} by the pixel time (0.78 msec) as well as converting Joules into photons (assuming 520 nm light) yields the total number of fluorescent photons emitted from the capillary during 1 pixel of data collection, $P_{out} \cong 5.2 \times 10^5$ photons/pixel.

Detected photons

The authors obtained 8.5 instrument counts at the LOD. This corresponds to 0.021 V. With a preamp gain of 10^7 V/A, this signal yields 2.1×10^{-9} A. The PMT gain is 2.65×10^1, and the conversion efficiency is 80 mA/W. These values lead to 9.8×10^{-13} W of fluorescent light entering the PMT. For 1 pixel of data collection, this corresponds to $P_D = 2.0 \times 10^3$ photons detected, giving an RQE for the instrument of:

$$RQE = \frac{P_D}{P_{out}} = 0.004 \qquad (2)$$

The number of instrument counts anticipated at the LOD can also be calculated from W_{out}:

$$Counts_{LOD} = W_{out}{}^{*}FE^{*}OBJ^{*}PMT\_EFF^{*}PMT\_CONV^{*}PMT\_G^{*}PA^{*}\left(\frac{4096\ counts}{10\ V}\right) \qquad (3)$$

where W_{out} (2.54×10^{-10} W) is defined as above, FE = filter transmission efficiency (0.58), OBJ = objective lens collection efficiency (0.056), PMT_EFF = PMT efficiency (0.18), PMT_CONV = PMT conversion efficiency (80 mA/W), PMT_G = PMT gain (2.65×10^4), and PA = preamp gain (10^7 V/A). Using the listed values, $Counts_{ION}$ = 13 cts is obtained, agreeing well with the 8.5 cts found experimentally.

DNA detection

DNA incubated with TO[16,17,24] was detected by flowing different concentrations of calf thymus DNA through the capillaries with 0.4 μ*M* TO included in the buffer (*Figure 7*). A linear response to DNA concentration was found over the range 0.03 ng DNA/μL to >1 ng DNA/μL. The limit of detection, defined at Signal = 2*(Background), was found to be 0.036 ng/μL.

Data analysis

The image analysis software, ArrayQuant, is based on the ImageQuant™ package developed at Molecular Dynamics. The data are collected in an image format, and analysis tools automate parts of the data processing. The 1-D capillary electropherograms are generated from the central 1 or 3 pixels (averaged) down each capillary in the image. Features in ArrayQuant include the subtraction of spectral cross-talk between the two optical channels, automatic generation of 1-D capillary electropherograms from the 2-D image format, peak finding, peak sizing of DNA fragments in base pairs, generation of result summaries, and export to Excel™ (Microsoft Corp., Bellevue, WA, U.S.A.) workbooks.

Operation process

After capillaries are installed in the unit, the interior glass surface is coated with a layer of polyacrylamide to eliminate electroendosmotic flow. The procedure is an adaptation of one developed by Hjertén[21] and takes approximately 4 hr. Once complete, the coating is robust and lasts for several weeks of continuous operation. The pressure manifold on the anode chamber is used to flush the necessary reagents through the capillaries under nitrogen.

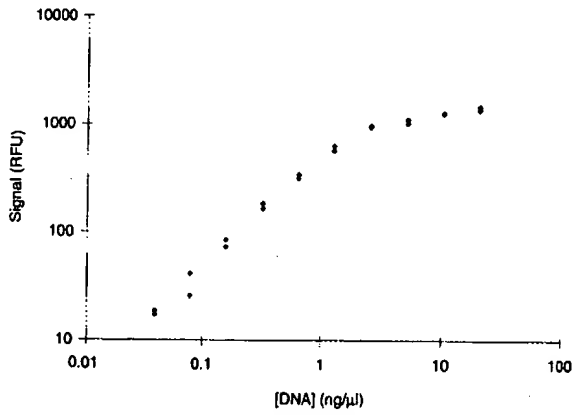

FIGURE 7 *Plot of signal* versus *DNA concentration. Capillaries filled with flowing calf thymus DNA solutions, TE buffer (pH 8.0), 0.4 μM TO, PMT 400 V.*

After the capillaries have been coated, they are flushed with TBE buffer and checked for electrical continuity at 2 kV with 48 individual buffer tubes mounted in the cathode sample rack. The anode buffer rack is then loaded with eight microcentrifuge tubes of sieving buffer, and N_2 pressure is turned on to pump sieving buffer into the capillaries. The fill time for the capillaries depends on the viscosity of the cellulose solutions. Typically, with a 0.5% HEC solution, the capillaries can be rinsed and refilled in 5 min, while a 1% HEC sieving buffer takes 20 min at 50 psi pressure. After the capillaries are filled with HEC, the cathode rack of TBE buffer is replaced with tubes containing the sieving buffer, and the system is prerun for 10–15 min at 10 kV.

Once the capillaries have equilibrated, the system is ready for sample injection. With the data acquisition system turned on and the high voltage turned off, the cathode rack holding the sieving buffer is removed. The outsides of the capillaries are then rinsed with a ddH_2O-filled tank. This step removes any HEC remaining on the outsides of the capillaries or held by surface tension between the capillaries and the electrodes, thereby improving the efficiency and uniformity of sample injections across the array. A rack holding sample tubes (e.g., dsDNA PCR products) is placed in position, and samples are then electrokinetically injected for 5–10 sec at 5 kV. The sample rack is removed, the sieving-buffer rack is placed in position, and the high voltage is reengaged. The entire sample injection process takes <30 sec for all 48 capillaries. Electrophoresis is routinely performed at 200 V/cm. The durations and current levels during sample injection and electrophoresis are stored by the current monitoring system, which is engaged whenever the data acquisition system is in operation. This information allows the accurate determination of retention times and electrical power curves for the capillaries. The optical data are presented on screen during the run, so that progress can be monitored. The current levels in all capillaries are also displayed, as is a time counter. Data collection may be stopped at any time, and no preset run time is required. After completion of an experiment (generally 30–40 min), the anode tubes are replaced with water or methanol, and the sieving buffer is expelled from the capillaries by applying N_2 pressure. For optimum performance, new sieving buffer is injected into the capillaries for each run. The ArrayQuant application is then used for data analysis. A full experimental turnaround time from one run to the next is approximately 1 hr, including electrophoretic separation. Capillaries rarely clog and do not break once installed in the unit. A capillary lifetime test subjected a fully loaded apparatus to approximately 60 runs over six weeks with no capillary failures.

Equipment and experimental conditions

The optical subsystem is shown in Figure 1 and described in detail in the Discussion section. The 20-mW Ar^+ laser (SL488) was purchased from Uniphase (San Jose, CA, U.S.A.). Optical filters and beamsplitters were obtained from Omega Optical (Brattleboro, VT, U.S.A.). The microscope objective was a Nikon 40× ELWD from Technical Instruments Corp. (San Francisco, CA, U.S.A.). Translation stage and controllers were purchased from Daedel (Harrison City, PA, U.S.A.). PMTs were Hamamatsu R1477-05 (Hamamatsu Photonics, Bridgewater, NJ, U.S.A.). BODIPY510/515 (ε_{488} = 14700) fluorescent dye was used. TO was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).

The electrophoresis subsystem is shown in Figures 2–4. A complete description is provided in the Discussion section. The electrophoresis voltage was supplied by a 20-kV power supply (Bertan, Hicksville, NY, U.S.A.). Tygon tubing was used. Data from the photomultipliers were read into a 12-bit DAS48PGA board, while capillary current information was collected by a 12-bit DAS16/330 board, both from ComputerBoards, Inc. (Mansfield, MA, U.S.A.). Images and current data were analyzed with ArrayQuant, a software package developed in-house that runs under the Windows NT™ (Microsoft Corp.) operating system.

Standard DNA samples consisted of 100-bp and 1-kbp double-stranded size ladders (Life Technologies Gibco BRL, Gaithersburg, MD, U.S.A.), the pGEM™ ladder supplied by Promega (Madison, WI, U.S.A.), or the 16-bp AmpliFLP™ D1S80 allelic ladder from Perkin-Elmer (Norwalk, CT, U.S.A.). Samples were diluted in Milli-Q™ (Millipore, Bedford, MA, U.S.A.) purified water, to a concentration of 0.1–1 ng/μL. Samples generated by PCR were diluted in water and/or desalted by float dialysis on membrane-bottomed multiple-well plates (Millipore, Bedford, MA, U.S.A.) for 20 min prior to injection. Standard buffer solutions used were Tris-EDTA (TE, 10 m*M* Tris, 1 m*M* EDTA, pH 8) and Tris-borate-EDTA (TBE, 89 m*M* Tris, 89 m*M* boric acid, 2 m*M* EDTA, pH 8).

The sieving buffer consisted of hydroxyethylcellulose (HEC, 90,000–105,000 MW, Polysciences, Warrington, PA, U.S.A.) at 0.5–1.5% wt/vol, 1 × TBE buffer (pH 8.0), and 0.4 μ*M* thiazole orange (Aldrich, Milwaukee, WI, U.S.A.). The HEC was dissolved in buffer overnight, then filtered and degassed under vacuum for 1 hr. The sieving buffer was then dispensed into 1.5-mL microcentrifuge tubes, thiazole orange was added, and the tubes were centrifuged for 10 min at 14,000 rpm. These tubes were then placed in the anode pressure manifold (Figure 4) for introduction of the sieving buffer into the capillaries.

The 48 capillaries (360-μm o.d., 100- or 75-μm i.d., Polymicro Technologies, Phoenix, AZ, U.S.A.) were cut to 65 cm in length. A detection window was created by burning away a 1-cm portion of the polyimide coating using a hot wire (Euramark, Mt. Prospect, IL, U.S.A.). The detection window, placed to give a 41-cm detection length, was cleaned carefully with methanol. The capillaries were then installed in the unit and a final 1.0 cm trimmed away from each end, giving a final run length to the detector of (L_d) 40 cm and a total capillary length (L_T) of 63 cm.

To eliminate electroendosmotic forces, the interior glass was coated with polyacrylamide using the procedure of Hjertén[21] as follows: The capillaries were first rinsed with 1 *M* NaOH for 45 min under low N_2 pressure, followed by water for 5 min and, finally, acetonitrile for 5 min. Next, 80 μL of γ-methacryloxypropyltrimethoxysilane (Petrarch Systems, Inc., Bristol, PA, U.S.A.) was added to 20 mL acetonitrile with 30 μL acetic acid. This solution was flowed slowly through the capillaries under N_2 pressure for 2 hr. The capillaries were then rinsed again with acetonitrile. A degassed 3% acrylamide solution was prepared in water, and *N,N,N'N'*-tetramethylethylenediamine (TEMED, 5 μL/mL of acrylamide solution, Eastman Kodak, Rochester, NY, U.S.A.) and ammonium persulfate (APS, 1 mg/mL of acrylamide solution) were added. The acrylamide was then pumped slowly through the capillaries under N_2 pressure over 45 min. The capillaries were subsequently rinsed with water and dried under N_2. Sodium hydroxide (reagent grade), APS, and acetonitrile (HPLC

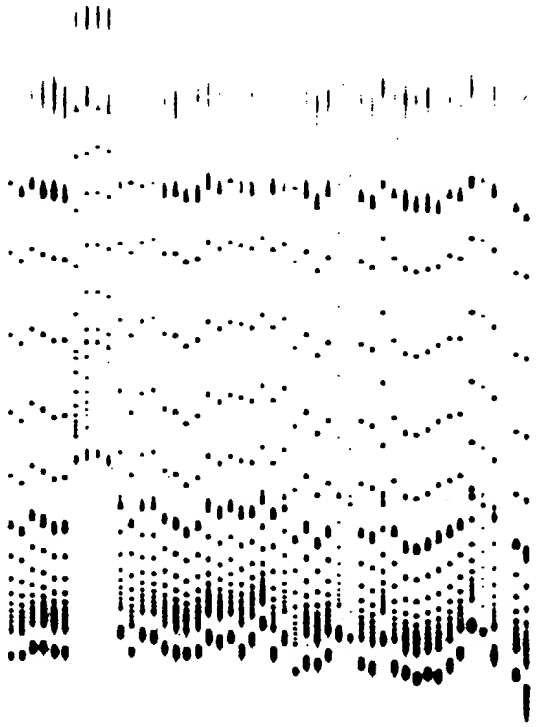

FIGURE 8 *Image of 48-capillary array. Sieving buffer: 1% wt/vol HEC, 0.5 × TBE, 0.4 μM TO. DNA: 100 bp dsDNA ladder. Sample injection: 5 sec at 5 kV. Electrophoresis: 10 kV (160 V/cm). Capillaries 360-μm o.d., 100-μm i.d., except #B1–B4: 360-μm o.d., 75-μm i.d.*

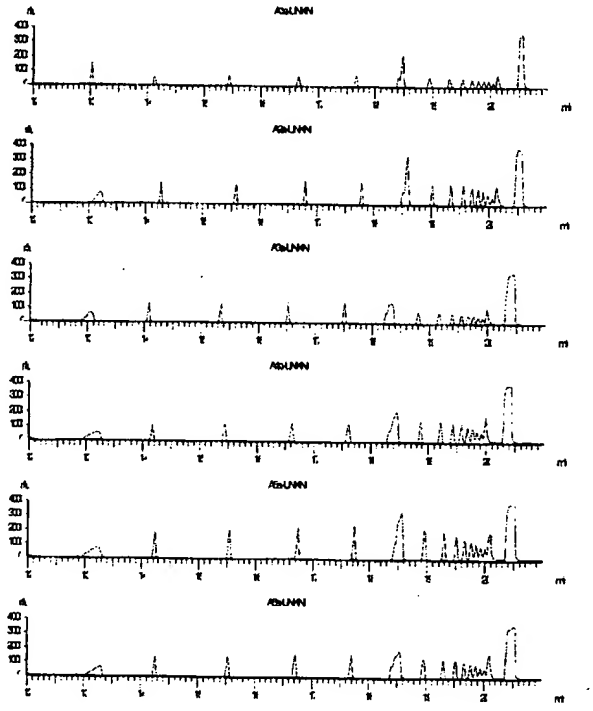

FIGURE 9 *Electropherograms of capillaries A1–A6 from Figure 8, showing fluorescence intensity versus time.*

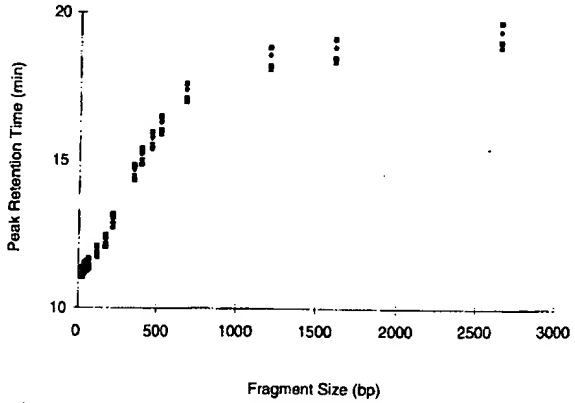

FIGURE 10 *Plot of peak retention time versus fragment size for dsDNA. Sieving buffer: 0.5% wt/vol HEC, 1× TBE, 0.4 μM TO, PMT 400 V. Electrophoresis: 155 V/cm. Sample: pGEM dsDNA ladder. Data shown for a group of eight capillaries from one instrument run.*

grade) were obtained from Sigma Chemical (St. Louis, MO, U.S.A.).

Instrument performance

Figure 8 shows a representative image, obtained using 1% HEC/1 × TBE, 0.4 μ*M* TO buffer, and a 100-bp DNA size ladder sample. Capillaries 1–48 are arranged from left to right in the image. The vertical axis is time, with short fragments appearing at the top of the image and longer fragments appearing later. The electropherograms for a subset of the capillaries are shown in *Figure 9*. From these peak identifications, ArrayQuant calculates peak retention times, peak standard deviations, and other statistical parameters. These data are then output to an Excel workbook.

The uniformity across the capillary array is quite high. Fluctuations in peak retention times are due to residual thermal variations across the capillary array. These differences result in variations in retention times of a few seconds, out of a total run time of approximately 40 min. Although these variations are small, accurate DNA sizing requires an internal standard for each capillary. The two-color capability of the instrument allows standards and unknowns to be end-labeled with different fluors. The authors have been able to size dsDNA PCR fragments (100–450 bp) using the 1% HEC/TO sieving buffer system, with a total error of <1% relative to sizes obtained by DNA sequencing.[26] The HEC sieving buffer provides high-quality separations over a wide range of DNA fragment sizes.[14–18,20,27–28,30–31,33,35–36,38–40] A 0.5–1.5% wt/vol solution of the polymer that yields effective separations of fragments between 100 and 2000 bp (*Figure 10*) is generally employed. By manipulating the concentration and molecular weight distribution of the sieving buffer, one can extend the range of useful separations. This flexibility increases the field of applications for which the CAE instrumentation is appropriate. The only limitation on applications for this generation instrument is the viscosity of the sieving buffer that can be pumped into the capillaries using the current pressure manifold design.

Conclusions

The system described here is an extension of the work done by Mathies et al.[1–3,16] The authors have developed a multicapillary scanning device that employs LIF for detection. The system has been designed to provide maximum flexibility and convenience for the user. Capillaries are individually replaceable, a pressure manifold permits the introduction of moderately viscous sieving buffers, and the detector design allows the use of any combination of 488-nm excitable fluors. No matrix polymerization or gel pouring is required, and all 48 samples are injected simultaneously in 5–15 sec. Sizing of dsDNA fragments can be accomplished quickly and with high accuracy and precision.[26] The instrument, therefore, represents a significant step on the path to a fully automated, very high throughput DNA analysis instrument aimed at large-scale DNA mapping, genetic typing, and DNA sequencing projects.

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